



Remineralizing Potential of Bioactive Glass–Ceramic over White Spot Lesions

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Abstract

Objective This study evaluated the effect of incorporating biosilicate (Bio) particles into experimental toothpaste (ET) on their abrasiveness and remineralization capacity for white spot lesions (WSLs).

Materials and Methods Thirty-two fragments of bovine teeth (6 × 6 × 2 mm) were obtained. Initial microhardness (Knoop hardness number [KHN], HMV Microhardness Meter, Shimadzu) and surface roughness (Rugosimeter Surfcorde SE 1700) readouts were performed. Fragments were submitted to a cariogenic challenge to simulate WSLs and then divided into four groups: Control, conventional toothpaste (Colgate Smiles, Colgate-Palmolive Company); ETF, ET with fluoride (carboxymethylcellulose + glycerol + thickening silica + fluoride); ETB, ET with Bio; BS, biosuspension (10 in weight% Bio). Toothpaste treatments were performed through simulated toothbrushing (Pepsodent, MAVTEC, 14,600 cycles). BS was applied by immersion for 8 hours followed by 16 hours in artificial saliva at 37°C for 60 days, totalizing 1,440 cycles. After treatments, final KHN and surface roughness readings were performed, and scanning electron microscopy (SEM) was conducted (Jeol JSM-6610LV) for morphological analysis. Data were analyzed with one-way analysis of variance, Tukey's test ($p < 0.05$).

Results BS produced the least surface roughness change, different ($p < 0.05$) from all the other groups. ETB caused higher KHN than ETF ($p < 0.05$). SEM images revealed that ETB and BS resulted in abraded surfaces with deposition of particles.

Conclusion ETB resulted in similar abrasiveness to the control group, and it caused higher microhardness than the ETF.

Practical Implication Considering its higher KHN, ETB could be considered a proper alternative for the treatment of WSLs.

Keywords

- ▶ toothpaste
- ▶ biomaterial
- ▶ biosilicate
- ▶ white spot lesion
- ▶ dental remineralization

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Introduction

The surface of dental enamel is often exposed to changes in pH that occur in the oral cavity. These changes are related to bacterial¹ and nonbacterial acid exposure (intrinsic and extrinsic),² which progress toward the dissolution of hydroxyapatite, resulting in caries and dental erosion, respectively.^{1,2}

Dental caries happen when the remineralization phase cannot control demineralization.^{3,4} *Streptococcus mutans* and other species involved in the etiology of caries produce large amounts of lactic acid in the presence of fermentable sugars, causing loss and impairment of tooth structure.^{5,6} The initial stage of demineralization results in white opaque areas, known as active white spot lesions (WSLs).⁷

WSLs are seen as white spots without cavity formation and are characterized by subsurface demineralization with an intact surface layer of enamel. These lesions are frequent in children and are associated with bad oral health habits and high sugar intake.⁸ Sometimes oral hygiene is not performed after nighttime feeding, increasing the risk of caries.⁸

The use of fluorides is the most studied strategy to strengthen dental enamel and is related to lower white spot prevalence.⁹ They prevent caries, inhibit demineralization, reinforce remineralization, and reduce the metabolic activity of bacteria and the enamel permeability.^{10,11} However, one of the limitations in their use is that fluorides have limited effectiveness in preventing mineral loss due to caries disease. The calcium fluoride layer formed during topical application tends to be gradually dissolved by acidic activity from the diet.¹²

So, there is a need to look for at-home alternatives that can arrest the progression of caries and induce remineralization of WSLs. Bioactive glasses have been investigated as remineralizing agents.¹³ These materials are capable of chemical bonding to dental hard tissues and are composed of calcium, sodium, phosphorus, and silica oxides that promote their bioactivity.¹⁴ These biomaterials can be applied directly to dental tissues and form a hydroxyapatite layer on mineralized tissues to promote remineralization.^{13–16} They have also been incorporated into toothpaste to determine their remineralization potential, showing promising results.¹⁴

Biosilicate (Bio), a bioactive glass ceramic with fully crystallized particles, has been introduced to combine the high mechanical bioactivity of glass with the strength of glass ceramics.^{12–14} It has also shown promising results on sound, and caries-affected dental tissues applied as suspension or air-abrasion.¹⁶ Considering that one of the first chemical reactions that occur when using Bio in the presence of fluids is the increase in pH, its use as a therapeutic agent to control oral pH and the presence of biofilm could help maintain oral health, in addition to reduce the incidence of caries, especially in cases where sucrose intake is frequent.¹²

However, little is known about the effect of this additive on dental enamel remineralization when incorporated into toothpaste, one of the most efficient ways to deliver therapeutic agents to tooth surfaces. Thus, the aim of this in vitro study was to evaluate the effect of incorporating Bio particles into experimental toothpaste (ET) on their abrasiveness and remineralization capacity for WSL. The null hypotheses

tested were that there would be no significant differences among the treatments in terms of (1) their abrasiveness or (2) their remineralization capacity.

Materials and Methods

Sample Preparation

The sample size calculation was performed after a pilot study comparing the mean values (www.openepi.com), with a confidence interval of 95% and sample power of 80%.

Sound bovine teeth were selected and cut into 32 fragments of 6 mm × 6 mm × 2 mm (metallographic precision saw Isomet 100 Buehler, Illinois, United States). Buccal faces were planned and polished with 600- and 1,200-grit silicon carbide sandpaper strips to remove grooves, and the surface roughness was standardized ($\leq 0.2 \mu\text{m}$).

Cariogenic Challenge

The samples were submitted to cariogenic challenge to simulate enamel demineralization in WSLs. The protocol was performed with 8% methylcellulose gel and lactic acid. The dentin of the fragments was protected with cosmetic nail polish (Colorama, Loreal Brasil, Rio de Janeiro, RJ, Brazil), and 1.5 mL of gel was added over it (pH = 4.6), which remained in contact with the surface of the fragment for 12 hours in a cold chamber (4°C). After this period, 1.5 mL of 0.1 M lactic acid (pH = 4.6) was added.¹⁷

After the cariogenic challenge, the samples were separated into four groups ($n = 8$) according to the treatment performed (►Table 1).

Obtaining the Toothpaste

Two similar formulations of ET based on carboxymethylcellulose (CMC) were obtained as described in ►Table 1. CMC was dissolved in 5 wt% of glycerol at 95°C, and the other components were added in the necessary proportion to obtain the proper consistency. One of the toothpastes had 1,450 ppm of sodium fluoride, and the different formulation was obtained by adding 10 wt% of Bio powder (average size of 4 μm) immediately before use, manually mixed with a spatula, until homogeneous distribution was achieved without separation of phases between the thickener and Bio.

Both ETs were compared with a commercial toothpaste (Colgate Smiles Batman and Mulher Maravilha, Colgate-Palmolive Company, São Bernardo do Campo, SP, Brazil) and the same concentration of Bio in suspension form after the addition of 10 wt% of Bio microparticles in distilled and deionized water, prepared immediately before application.

pH Test

Before the treatments, the pH of the toothpaste and the BS was measured using a digital pH meter (Kasvi model K39-2014B, Paraná, Brazil) calibrated with standard solutions.¹⁸

Simulated Brushing

For the treatment with the toothpaste, the samples were submitted to brushing in a toothbrushing simulator

Table 1 Experimental groups

| Groups | Products | Composition |
|-----------------|---|--|
| G1 (Control) | Colgate Smiles toothpaste (Batman and Wonder Woman) 100 mg | Sodium fluoride 0.242%, Sorbitol, Water, Hydrated silica, PEG-12, Cellulose gum, Sodium lauryl sulfate, Flavor Sarcarina sodica, Sodium fluoride, Mica (CI77019), Titanium dioxide (CI77891), FD&C Rojo No. 40 9CI1603 |
| G2 | Experimental toothpaste with fluoride | Carboxymethylcellulose (2.0 g), Glycerin (74.68 g), Tixosil 73 (10.0 g), Tixosil 43B (13.9 g), Sodium fluoride (1,450 ppm) |
| G3 | Experimental toothpaste with biosilicate | Carboxymethylcellulose (2.0 g), Glycerin (74.68 g), Tixosil 73 (10.0 g), Tixosil 43B (13.9 g), Biosilicate 10% (5.0 g) |
| G4 | Biosilicate suspension (10%) in distilled and deionized water | Distilled and deionized water (20 mL) and biosilicate (10% by weight) |

(Pepsodent, MAVTEC Comércio De Peças Acessorios E Serviços Ltda Me, Ribeirão Preto, SP, Brazil). A soft children's toothbrush (Tek, Johnson & Johnson Ind. Com. Ltd., São José dos Campos, SP, Brazil) was used for each sample. The brush heads were fitted and fixed in the machine. The fragments were submitted to 14,600 brushing cycles under a constant load of 200 g and a traveled course of 3.8 cm at a speed of 356 rpm, simulating 1 year of brushing by a healthy individual.¹⁹ The samples were fixed in acrylic resin plates (Acrilpress Artefatos de acrylic Ltda, Ribeirão Preto, SP, Brazil) with hot glue, allowing immobilization of the samples at the time of brushing.

The toothpastes were diluted in a proportion of 1:1 in distilled water immediately before use to obtain a slurry with uniform consistency. For each sample, 10 mL of the slurry was poured, and the fragments were brushed.

Suspension Immersion

After the addition of the Bio powder in distilled and deionized water, the suspension was vigorously shaken for 3 minutes, and then, 1.5 mL of the BS was pipetted into Eppendorf. The samples were immersed in the suspension for 8 hours, and after that, they remained in artificial saliva for 16 hours at 37°C. This treatment was performed for 60 days, making up 1,440 cycles.

Surface Roughness

Surface roughness analysis (Rugosimeter SE 1700 Surfcorde, Kosakalab, Tokyo, Japan) was performed before and after treatments, with three cutoff lengths of 0.8 mm, totalizing a reading length of 2.4 mm, at a speed of 0.25 mm/s.

Three readings were taken at different locations on the surface of the samples, perpendicular to the brushing direction: one central, one 1 mm to the right, and one 1 mm to the left. The average of these values was used as surface roughness measurement. The surface roughness variation was calculated by the difference between the final and initial values.

Microhardness Analysis

Knoop microhardness number (KHN) analysis (Microhardness Tester HMV-2, Shimadzu, Tokyo, Japan) was performed with a vertical static load of 25 g applied for 5 seconds at 40× objective. When activated, the penetrator tip compresses the

surface of the pattern, generating a geometric figure in the form of an inverted pyramid. The diamond makes it possible to determine the surface KHN of the material from the measurement of its largest diagonal, whose value is applied in the formula:

$$KHN = 1,451 \frac{F}{d^2}$$

where

KHN, Knoop hardness number;

F = 25 g;

d = length of the longest diagonal in the indentation.

Five different readings were performed on the samples, each one equidistant at 1 mm from each other. The average of the five readings was considered as the KHN value. The KHN change (Δ KHN) was calculated considering the relative differences in relation to the initial values by the formula:

$$\Delta KHN = \left(\frac{KHN_f - KHN_i}{KHN_i} \right) \times 100$$

Where Δ KHN is the relative KHN calculated, KHN_i is the initial value, and KHN_f is the final value obtained. The remineralizing potential of the treatments applied was also calculated using the final values and the values obtained after the cariogenic challenge by the formula:

$$\text{Potential} = \frac{(KHN_f - KHN_c)}{(KHN_i - KHN_c)} \times 100$$

Where KHN_c corresponds to the KHN value after the cariogenic challenge.

Scanning Electron Microscopy

Qualitative analyses of the enamel surface were performed. Two samples were randomly selected from each group, which were observed under scanning electron microscopy (SEM; Jeol JSM-6610LV Microscope, Sony, Tokyo, Japan) to compare the sound and treated enamel. The fragments were fixed on aluminum stubs and sputter-coated with gold-palladium alloy (Bal-Tec, model SCD 050 sputter coater, Balzers, Liechtenstein). The surfaces were analyzed at 500×, 1,000×, and 2,000× magnifications (20 kV, 30 mm working distance [WD], and spot size 28 mm).

Table 2 Comparison of means (standard deviation) of surface roughness change, relative microhardness, and remineralizing potential between groups

| | Control | Fluoride toothpaste | Biosilicate toothpaste | Biosilicate suspension |
|-------------------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| Surface roughness | 0.70 (0.23) ^a | 0.34 (0.25) ^b | 0.45 (0.15) ^{ab} | 0.06 (0.04) ^c |
| Relative microhardness (Percentage) | 75.3 (5.1) ^{ab} | 66.3 (12.8) ^b | 78.9 (8.3) ^a | 75.2 (4.9) ^{ab} |
| Remineralizing potential | 33.1 (15.7) ^a | 55.0 (22.5) ^a | 54.4 (20.9) ^a | 55.3 (17.8) ^a |

Different superscript letters, between the columns, indicate a statistically significant difference.

Statistical Analysis

Quantitative data were analyzed according to their distribution using the Shapiro–Wilk test, with a significance level of 95% ($\alpha = 0.05$). Data distribution was considered normal for all the parameters analyzed and, therefore, they were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test, with a significance level of 95%.

Results

pH

pH values of the treatments were 7.6 for Control, 7.34 for experimental toothpaste with fluoride, 8.4 for experimental toothpaste with biosilicate, and 9.5 for 10% BS.

Surface Roughness

The surface roughness means and their comparisons (one-way ANOVA, Tukey's test, $p < 0.05$) can be seen in ►Table 2. Control group showed more remarkable surface roughness alteration ($p < 0.05$) than the groups treated with the fluoride toothpaste and the BS presented the slightest alteration, different from all the other groups ($p < 0.05$). The toothpaste containing Bio resulted in surface roughness alteration similar to the Control and the group treated with the fluoride toothpaste ($p > 0.05$).

Microhardness Analysis

The comparison of the relative KHN means (percentage) is shown in ►Table 2. The treatments were not able to fully recover the initial KHN of the enamel after demineralization. There was no difference between the groups ($p > 0.05$), except when comparing the fluoride toothpaste and the one with Bio ($p < 0.05$). The fluoride toothpaste had the lowest relative KHN values but similar ($p > 0.05$) to the Control and the group treated with the BS.

The mean comparison of the remineralizing potential (Kruskal–Wallis, Dunn's test, $p < 0.05$) is presented in ►Table 2. There was no difference between the groups ($p > 0.05$).

Scanning Electron Microscopy

Representative SEM images are shown in ►Fig. 1. Sound enamel surfaces showed a scratched appearance after grinding with sandpaper. The Control group (brushed with conventional toothpaste) demonstrated a more polished enamel surface than the sound enamel. The samples brushed with the fluoride toothpaste had an enamel surface with a reticu-

lar structure. At higher magnification, an abraded and irregular enamel surface was observed. The samples brushed with the toothpaste containing the Bio revealed a more polished enamel surface than the sound enamel and Bio particles on the surface. The samples treated with the BS also presented grooves and scratches on the surface resulting from the polishing process. In addition, Bio particles were also seen on the surface, and at higher magnification, it is possible to verify regions of remineralization.

Discussion

This study aimed to evaluate the impact of incorporating 10% Bio into an ET on their abrasiveness and ability to remineralize artificially created WSL. The ET containing Bio particles was compared with a conventional children's toothpaste (Control) and regularly marketed,^{8,9} and an ET with 1,450 ppm of fluoride, that was also included to isolate the influence of other ingredients on dental enamel. The effectiveness of applying a 10% BS was also tested, since previous studies have proven its remineralizing capacity.^{17,20}

The first null hypothesis, which posited similar abrasiveness among treatments, was rejected, based on the study results ($p < 0.05$). The conventional toothpaste was significantly more abrasive than the other treatments, causing greater surface roughness compared with the ET with fluoride and BS, which had little impact on surface roughness of the enamel.²¹

Commercial toothpastes formulations typically contain abrasives for stains and biofilm removal.^{22,23} However, these abrasives may also cause enamel wear, gradually increasing the surface roughness of the enamel over time.²² SEM analysis showed linear scratches on untreated enamel surfaces due to sandpaper polishing. Interestingly, after toothbrushing with the conventional toothpaste, the enamel surface appeared more polished, demonstrating that the abrasives can indeed contribute to enamel wear.²¹

The nature of abrasives, including type, quantity, and particle size, significantly influences a toothpaste's abrasiveness.²³ The conventional toothpaste contained hydrated silica as its abrasive component,²⁴ known for its potential to induce dental wear.²⁵ According to Ali et al 2020,²⁵ hydrated silica's higher Mohs hardness scale value compared with enamel (ranging from 2.5 to 5 vs. 3.5) can lead to enamel surface scratches and increased roughness. Furthermore, in the present study, we simulated incipient caries lesions (WSLs) by inducing superficial and subsurface demineralization of the

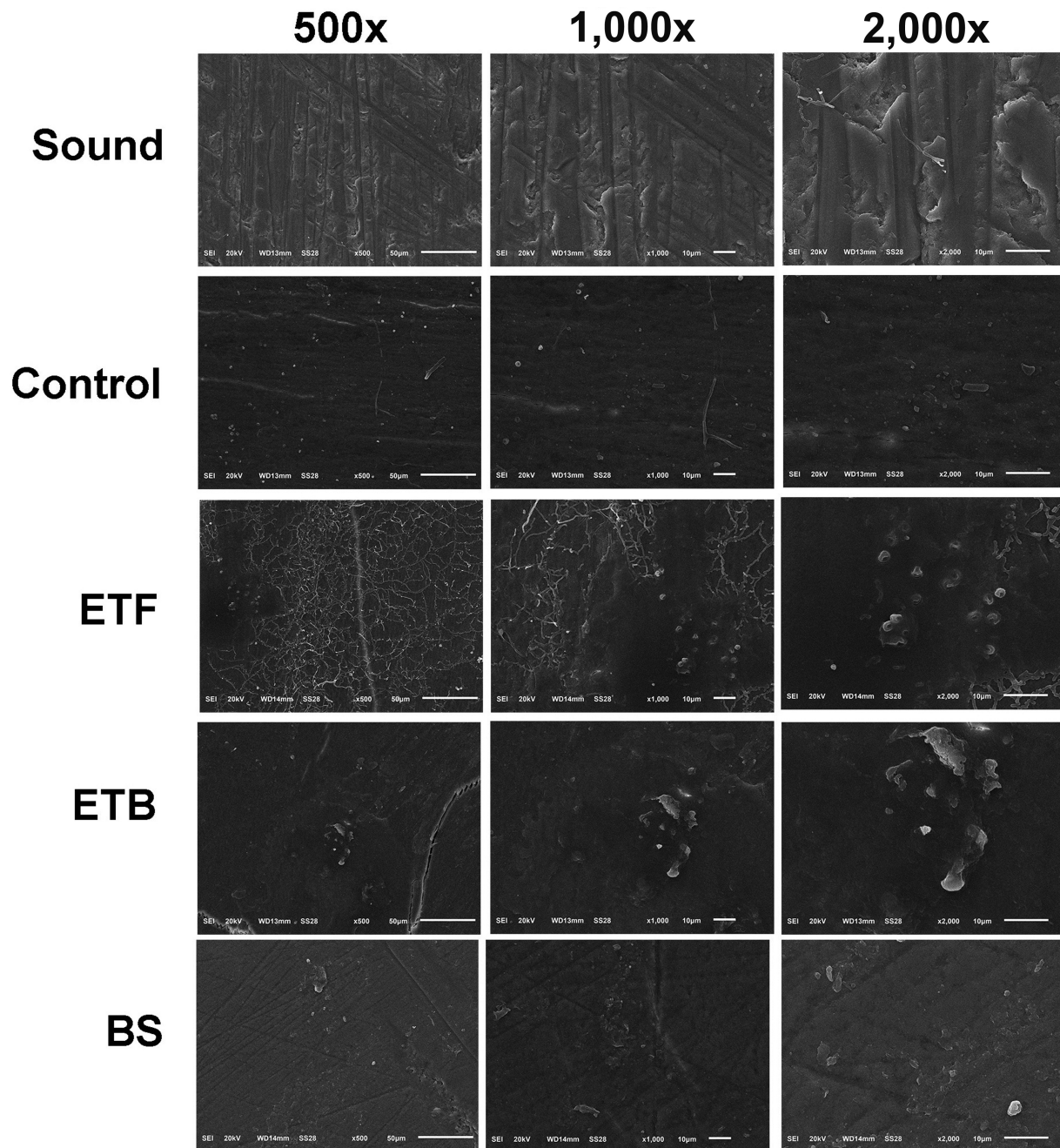


Fig. 1 Representative scanning electron microscopy images of each group. Images were obtained at three magnifications: 500 × , 1,000 × , and 2,000 × . BS, Biosuspension; ETB, experimental toothpaste with biosilicate; ETF, experimental toothpaste with fluoride.

enamel surface. Due to the mineral loss, the affected lesion surfaces become softer and more susceptible to abrasion from toothbrushing.²³

In contrast, the ET with fluoride functioned solely as a vehicle for delivering the therapeutic agent and did not include abrasive particles, which could explain its low abrasiveness. Moreover, the fluoride in this toothpaste may have prevented the dissolution of calcium hydroxyapatite,²⁶ helping to maintain the surface roughness. SEM observations showed sediments on the enamel surface, suggesting the formation of a filamentous network due to the deposition of fluoride, which is insoluble in glycerin. Additionally, despite

dilution in water to create the slurry, sodium fluoride is poorly soluble in water.²⁷

The ET with Bio showed intermediate surface roughness results, attributed to the presence of Bio particles with an average size of 4 µm. Bio particles are highly bioactive and undergo a rapid multistage surface reaction when in contact with an aqueous media, ultimately resulting in the formation of hydroxycarbonate apatite.¹⁵ Therefore, ET with Bio was not formulated with water or simulated body fluid; instead, it was formulated with CMC and glycerin as a vehicle²⁸ and was exposed to water only immediately before the simulated brushing.

Furthermore, despite their high bioactivity, Bio particles take some time to completely dissolve, as observed in a previous study²⁹ and confirmed in our study through SEM analysis. The simulated brushing lasted for 41 minutes, which appeared to be insufficient time for their complete dissolution. Consequently, the partially dissolved Bio particles may have acted as abrasives. However, this abrasive effect might have been mitigated by the remineralization process, resulting in intermediate results.

In contrast, the treatment with BS involved immersion, which explains the low surface roughness alteration and the presence of some grooves due to the polishing process, as no simulated toothbrushing occurred.

The second null hypothesis, regarding remineralizing potential, was accepted as there were no significant differences among the treatments. The most common and efficient strategy for reducing enamel demineralization is toothbrushing with a fluoride toothpaste.^{11,30,31} Fluoride replaces the hydroxyl ions in apatite structure, forming fluorapatite. This process enhances the enamel hardness, as demonstrated in the present study, and makes it more acid resistant.^{31–35} According to Toti et al (2022), daily fluoride toothpaste use has been associated with reduced WSL prevalence in children.⁸

While fluoride prevents dental demineralization, Bio acts as a remineralizing agent by inducing the formation of a silica gel-rich layer on the dental surfaces. This layer facilitates the deposition of calcium and phosphate ion, creating an amorphous calcium phosphate (ACP) layer. Over time, this ACP layer undergoes crystallization and transforms into hydroxy carbonate apatite (HCA).¹⁵ Thus, Bio promotes mineral replacement, while fluoride protects existing minerals.

A previous study by Ferreira et al (2022), revealed that the treatment of caries-affected dentin with 10% BS and silver diamine fluoride yielded comparable microhardness values.³⁶ This suggests that both treatments have a similar remineralizing capacity. Other studies have also reported similar efficiency in remineralizing initial lesions when comparing a fluoride toothpaste to a fluoride-free toothpaste containing microcrystalline hydroxyapatite.^{24,37–39} However, Chinelatti et al 2017, found that Bio has demonstrated superior and continuous remineralization compared with topical fluoride application.¹² Notably, our study assessed the incorporation of Bio into toothpaste, a novel approach.

Despite similar remineralizing capacity among treatments, enamel treated with Bio-containing ET exhibited higher relative KHN values compared with fluoride-containing ET ($p < 0.05$). Toothpaste pH influenced the efficiency of these remineralizing agents. A lower pH in fluoride-based agents promotes calcium fluoride formation,^{34,35} as observed in a previous study where reducing the pH of the toothpaste enhanced the fluoride uptake in the dental biofilm.^{35–37} In this study, both commercial and experimental fluoride toothpastes had a neutral pH (7.6 and 7.3, respectively), potentially limiting fluoride's effectiveness.

Conversely, the ET with Bio had a basic pH (8.4) due to the initial reaction between the glass–ceramic and aqueous media. This reaction leaches alkaline ions from the glass¹⁵

resulting in a basic pH that favors the formation of the silica gel-rich layer. Thus, in this case, the pH was favorable for remineralization.

Toothbrushing with fluoride toothpastes leads to immediate high fluoride levels on enamel surfaces,³⁷ but its effectiveness is limited since salivary flow rapidly washes away the deposited fluoride.³⁸ In contrast, Bio particles, with their high bioactivity, rapidly form an HCA layer similar to natural enamel mineral, explaining their superior KHN values.

The BS had a more basic pH (= 9.5) due to direct dissolution in water, accelerating the reaction rate. However, excessively high reaction rates can hinder the formation of the silica gel-rich layer, reducing ion uptake, and consequently, remineralization.³⁹ This likely explains why BS resulted in a similar relative KHN to fluoride toothpastes, rather than a higher relative KHN as observed with ET containing Bio.

In conclusion, all evaluated treatments showed similar remineralizing capacity. However, both Bio and fluoride have limitations. Bio requires time to initiate the remineralization process, while fluoride, although readily available, is swiftly washed away from the enamel surface.

One limitation of our study is that the ET with fluoride lacked water, potentially limiting its efficiency. Further studies should explore the effectiveness of these toothpaste formulations, including variations in concentrations and particle sizes of abrasives and Bio. Additionally, investigating the synergy between Bio and fluoride could yield valuable insights, as prior studies suggest potential benefits, such as fluoride release in the oral cavity and potential anticarcinogenic properties.^{40,41}

Conclusion

Within the limitations of the study, we can conclude that the toothpaste incorporating 10% Bio particles exhibited comparable abrasiveness to the commercial toothpaste. While there was no disparity in remineralization potential between the treatments, it is worth noting that the KHN of dental enamel following treatment with the experimental Bio-containing toothpaste exceeded that of the fluoride-based toothpaste. Consequently, the Bio-infused toothpaste may be a viable option for addressing WSL in children.

Funding

None.

Conflict of Interest

None declared.

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